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Augmentation of methamphetamine-induced behaviors in transgenic mice lacking the trace amine-associated receptor 1

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Abstract

The trace amine-associated receptor 1 (TAAR1) is a G protein-coupled receptor that is functionally activated by amphetamine-based psychostimulants, including amphetamine, methamphetamine and MDMA. Previous studies have shown that in transgenic mice lacking the TAAR1 gene (TAAR1 knockout; KO) a single injection of amphetamine can produce enhanced behavioral responses compared to responses evoked in wild-type (WT) mice. Further, the psychostimulant effects of cocaine can be diminished by selective activation of TAAR1. These findings suggest that TAAR1 might be implicated in the rewarding properties of psychostimulants. To investigate the role of TAAR1 in the rewarding effects of drugs of abuse, the psychomotor stimulating effects of amphetamine and methamphetamine and the conditioned rewarding effects of methamphetamine and morphine were compared between WT and TAAR1 KO mice. In locomotor activity studies, both single and repeated exposure to *d*-amphetamine or methamphetamine generated significantly higher levels of total distance travelled in TAAR1 KO mice compared to WT mice. In conditioned place preference (CPP) studies, TAAR1 KO mice acquired methamphetamine-induced CPP earlier than WT mice and retained CPP longer during extinction training. In orphine-induced CPP, both WT and KO genotypes displayed similar levels of CPP. Results from locomotor activity studies suggest that TAAR1 may have a modulatory role in the behavioral sensitization to amphetamine-based psychostimulants. That methamphetamine- but not morphine-induced CPP was augmented in TAAR1 KO mice suggests a selective role of TAAR1 in the conditioned reinforcing effects of methamphetamine. Collectively, these findings provide support for a regulatory role of TAAR1 in methamphetamine signaling.

Keywords

conditioned place preference; rewarding effects; morphine; psychomotor stimulating effects; dopamine; TAAR1

1. Introduction

Growing evidence suggests that trace amine-associated receptor 1 (TAAR1) may play a role in the behavioral effects of drugs of abuse (Berry et al. 2007, review). However, the

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influence of TAAR1 appears to be more contributory than causal and its mechanisms of action are complex and still not completely understood (Miller, 2011, review). In addition to common biogenic and trace amines, TAAR1 can be activated by amphetamine-based psychostimulants (Bunzow et al. 2001; Reese et al. 2007; Xie et al. 2007). TAAR1-positive cell bodies are located in brain regions implicated in mediating reward-related behaviors, including the ventral tegmental area, dorsal raphe nucleus and amygdala (Borowsky et al. 2001; Lindemann et al. 2007). Although β -PEA and amphetamine are different with respect to the role of dopaminergic mechanisms in their reinforcing properties and the lack of aversive effects in response to β -PEA (c.f. Greeshaw, 1984 and Risner and Jones, 1977), previous studies have demonstrated that endogenous trace amines such as β -phenethylamine (β -PEA) and tyramine can be as effective as amphetamine in their ability to maintain intravenous self-administration (Risner and Jones, 1977; Shannon and Thompson, 1984), decrease reward thresholds for self-stimulation of the lateral hypothalamus (Stein, 1964) and establish conditioned place preference (Gilbert and Cooper, 1983) in laboratory animals.

Trace amines (and amphetamines) are monoamine transporter substrates, suggesting that their capability to act as reinforcers could reside in their ability to raise extracellular monoamine levels via competitive inhibition of monoamine uptake (see Xie and Miller, 2009a for review). However, in addition to these competitive effects, non-competitive effects on monoamine transporter function resulting from TAAR1 activation have been observed. For example, activation of TAAR1 by β -PEA, methamphetamine, or common biogenic amines in the presence of specific monoamine autoreceptor antagonists, triggers phosphorylation-dependent monoamine transporter efflux in synaptosomes (Xie and Miller, 2008; 2009b; Xie et al; 2008). In addition, TAAR1 activation has been reported to decrease dopamine neuron firing activity and subsequent dopamine release (Lindemann et al. 2008; Bradaia et al. 2009) and block cocaine-induced hyperlocomotion (Revel et al. 2011). Alternatively, in mice lacking TAAR1 a single injection of amphetamine produces greater locomotor stimulating effects and higher levels of striatal dopamine release compared to wild-type (WT) littermates (Wolinsky et al. 2007; Lindemann et al. 2008). These differences between WT and TAAR1 KO mice in the locomotor stimulating effects of *d*-amphetamine along with several recent investigations of TAAR1 functionality in brain monoaminergic systems (reviewed in Miller, 2011) indicate that TAAR1 plays a regulatory role in the neurochemical and behavioral effects of *d*-amphetamine.

Based on these previous findings, the present study was undertaken to extend investigations into the role of TAAR1 in mediating reward-related behaviors of methamphetamine in comparison to morphine, a non-amphetamine drug with rewarding properties (van der Kooy et al. 1982). We first sought to confirm that the TAAR1 KO mice exhibited enhanced psychomotor stimulating effects in response to *d*-amphetamine exposure. Secondly, the role of TAAR1 in the psychomotor stimulating effects of methamphetamine was examined following acute and repeated methamphetamine exposure. We then investigated the extent to which TAAR1 is involved in drug reward by comparing the conditioned rewarding effects of methamphetamine to morphine in WT and TAAR1 KO mice using the conditioned place preference (CPP) paradigm.

2. Materials and Methods

2.1 Subjects

All subjects were male mice (approximately 10-12 weeks old; 28–32 g) that were maintained on a 12-h light/dark lighting schedule at a room temperature of $22\pm 1^{\circ}\text{C}$ and were individually housed with free access to food and water. TAAR1 KO and their WT counterparts were derived from heterozygous breeding pairs. The C57Bl-derived mice were a gift from Lundbeck Research USA, Inc. (Paramus, NJ) and their origination has been

previously been described (Wolinsky et al. 2007). Briefly, the TAAR1 knockout mice lack the single coding exon of the TAAR1 gene as well as 1.0 kb of upstream sequence and 1.3 kb of downstream sequence, which was replaced with a neo cassette in reverse orientation. Accordingly, the TAAR1 KO mice do not harbor a TAAR1 gene and do not express TAAR1 protein. All animal care was in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, National Academy Press, 1996) and all procedures were conducted in accordance with the Animal Experimentation Protocol #04184 approved by the Harvard Medical Area Standing Committee on Animals.

2.2 Drugs and chemicals

D-amphetamine sulfate, (+)-methamphetamine hydrochloride and morphine sulfate salt pentahydrate were purchased from Sigma (St Louis, MO). Drug solutions were dissolved in 0.9% saline solution and all drug treatments described below were administered intraperitoneally (i.p.) in volumes up to 0.1 mL/10 g body weight.

2.3 Apparatus

In all studies daily experimental sessions were conducted in ventilated, sound-attenuated chambers with white background noise (MED Associates, St. Albans, VT, USA) situated in a room separate from animal home cages. For locomotor activity experiments, the square open-field Plexiglas arena (11 cm × 11 cm) was equipped with three rows of infrared light-sensitive photocells mounted at 40, 115 and 195 mm above the floor. Each CPP arena was separated by a removable guillotine door into two compartments with distinct visual and tactile stimuli. One compartment comprised a floor made of parallel steel bars (1/4 inch apart) with four black walls while the other compartment comprised a white foam floor with four white walls. The arena was covered with a transparent lid perforated to allow adequate ventilation. Data collected from sensors were recorded and analyzed by the Activity Monitor MED-PC IV Interface.

2.4 Locomotor activity

2.4.1 Acute exposure—Each animal was habituated to the open-field arena for 30 min prior to drug administration. Different groups of WT and TAAR1 KO mice were administered i.p. injections of *d*-amphetamine, methamphetamine (1, 3 or 5 mg/kg) or saline. The total distance travelled over 60 min was recorded.

2.4.2 Repeated exposure—To compare the effects of repeated exposure to amphetamine and methamphetamine on locomotor activity, a separate group of WT and TAAR1 KO mice were administered escalating doses of *d*-amphetamine or methamphetamine (0.5, 1, 2, 3, 4, 5, 6 mg/kg) twice weekly for seven consecutive weeks and the total distance travelled following each injection was recorded for 60 min.

2.5 Conditioned place preference

As previously described in detail in Achat-Mendes et al. (2005), prior to conditioning, a separate group of naïve mice underwent a habituation phase in which the opened guillotine door allowed mice to explore the apparatus for 10 min. Seventy-two hours later, mice were again allowed access to the two chambers and the time spent in each compartment was measured for 20 min to evaluate potential bias for compartments. Mice spending ≥ 65% time in a particular compartment were eliminated. For each CPP experiment, about 15% of total mice were eliminated after the pre-conditioning test. Drug-paired compartments were randomly assigned to ensure that approximate numbers of animals within each genotype received pairings with drug in either the black compartment or the white compartment. During the conditioning phase, saline or the conditioning drug was administered and mice

were confined to the saline-paired or the drug-paired compartment, respectively. There were a total of 4 saline-paired and 4 drug-paired sessions during the conditioning phase. Control mice for both genotypes received saline in alternating compartments. Conditioning sessions took place throughout the day and were always performed at the same time of the day for each subject. To evaluate CPP, 20-min post-conditioning tests were conducted in which mice were allowed access to both compartments in the absence of drug or saline injections and time spent in each compartment was recorded. Total horizontal distance travelled was recorded during post-conditioning tests to compare locomotor activity between genotypes during testing.

2.5.1 Dose-response function of methamphetamine-induced CPP—The effects of a range of methamphetamine doses (0.3, 1.0 and 3.0 mg/kg) on the induction of CPP were evaluated in different groups of WT and TAAR1 KO mice. To test whether TAAR1 KO mice would be more sensitive to the conditioned rewarding effects of methamphetamine compared to WT mice, the 10-fold range of conditioning doses of methamphetamine included doses reported to induce weak to robust levels of place preference (c.f. Cunningham and Noble, 1992; Gehrke et al. 2003).

2.5.2 Acquisition, extinction and reinstatement of CPP induced by methamphetamine (1.0 mg/kg)—To further investigate differences in the sensitivity to the conditioned rewarding effects of methamphetamine between WT and TAAR1 KO mice, the intermediate methamphetamine dose (1.0 mg/kg) that produced the greatest CPP was used to evaluate conditioning under sub-optimal conditions, i.e. after only 4 conditioning sessions of 2 methamphetamine and 2 saline treatments. CPP testing was conducted as described above in which the first post-conditioning test took place after 2 methamphetamine conditioning sessions and the second post-conditioning test took place after 4 methamphetamine conditioning sessions. Extinction of methamphetamine-induced place preference was achieved by weekly exposure to both the drug- and saline-paired compartments in the absence of injections until no significant difference between the times spent in the drug- versus the saline-paired compartments was found. To compare the ability of methamphetamine to reinstate extinguished place preference between genotypes, a challenge dose of methamphetamine (0.5 mg/kg) was administered 24 hours following extinction and the time spent in both compartments recorded as in CPP tests.

2.5.3 Morphine-induced CPP—WT and TAAR1 KO mice were administered morphine (5 or 15 mg/kg) or saline and conditioning took place as described above. CPP was evaluated after 2 and again after 4 morphine sessions (Post-conditioning test 1 and test 2, respectively).

2.6 Data analysis

All data are presented as the mean $\pm$ SEM. Locomotor activity was expressed as total horizontal distance traveled during the session in cm. The CPP score was computed as a difference in time spent between drug-paired and saline-paired compartments. Data on effects of repeated exposure on locomotor activity were analyzed by repeated measures analyses of variance (RM ANOVA). All other data were analyzed by one-way or two-way ANOVAs for comparison between genotypes, treatments, or doses followed by post hoc Bonferroni's multiple comparison t-tests. A significance criterion of $p < 0.05$ was used for all analyses.

3 Results

3.1 Locomotor activity

3.1.1 Effects of acute exposure to d-amphetamine and methamphetamine on locomotor activity—For both amphetamine and methamphetamine, increasing the doses from 0-5 mg/kg produced an inverted U-shaped function in which the total distance traveled peaked in response to 3mg/kg and decreased when the highest dose (5mg/kg) was administered in both WT and TAAR1 KO mice (Figure 1). This characteristic shape of the locomotor effects of psychostimulants may be attributed to the marked increase in the mean number of vertical counts, i.e. rearing activity observed following 5 mg/kg compared to 3 mg/kg methamphetamine in WT mice (445 ± 253 versus 705 ± 594 , respectively). A two-way ANOVA of the total distance traveled following amphetamine administration revealed a main effect of genotype [$F(1,122)=5$; $p < 0.05$] and of drug dose [$F(3,122)=77$; $p < 0.0001$] and Bonferroni t-tests further revealed a significant increase in the total distance traveled between WT and TAAR1 KO mice following 3 mg/kg amphetamine (*: $p < 0.05$) for which the mean distance travelled was 29045 ± 3159 cm in WT mice compared to 38816 ± 4346 cm in TAAR1 KO mice. A two-way ANOVA of locomotor activity following administration of methamphetamine also revealed a significant effect of genotype [$F(1,122)=7$; $p < 0.01$] and a main effect of drug dose [$F(3,122)=75$; $p < 0.0001$]. As with amphetamine, the intermediate dose of methamphetamine produced significantly higher locomotor activity in TAAR1 KO mice (33850 ± 3034 cm) compared to WT mice (23897 ± 2199 cm); Bonferroni t-tests (**: $p < 0.01$).

3.2 Effects of repeated administration of d-amphetamine and methamphetamine on locomotor activity—Administration of escalating doses of both *d*-amphetamine and methamphetamine produced an inverted U-shaped dose-response curve with total distance traveled peaking in response to 2 mg/kg *d*-amphetamine and methamphetamine in WT mice (Figure 2). Two-way RM ANOVA of distance traveled following *d*-amphetamine administration revealed a significant effect of genotype [$F(1,24)=10$; $p < 0.05$], dose [$F(6,24)=24$; $p < 0.0001$] and genotype $\times$ dose interaction [$F(6,24)=2.8$; $p < 0.05$]. Similarly, a two-way RM ANOVA of locomotor activity following repeated methamphetamine treatments found a significant effect of genotype [$F(1,42)=10$; $p < 0.05$] and dose [$F(6,42)=41$; $p < 0.0001$]. Bonferroni t-tests further revealed a significant difference in total distance traveled between WT and TAAR1 KO mice following 3 mg/kg *d*-amphetamine and 2 mg/kg methamphetamine (*: $p < 0.05$).

3.3 Conditioned place preference

3.3.1 Methamphetamine-induced CPP—As illustrated in Figure 3A, the lowest (0.3 mg/kg) and highest (3.0 mg/kg) dose of methamphetamine produced low levels of place preference whereas the intermediate dose of methamphetamine (1.0 mg/kg) resulted in the largest preference for the methamphetamine-paired compartment. Two-way ANOVA of the difference in time spent in compartments revealed a significant effect of methamphetamine dose [$F(2,56)=5$; $p < 0.01$]. Bonferroni t-tests further found a significant difference in the magnitude of CPP for both WT and TAAR1 KO mice comparing conditioning with 1 mg/kg methamphetamine versus saline ($\#$: $p < 0.05$). Although there was no significant effect of genotype on the magnitude of CPP, [$F(1, 56) = 1$; $p < 0.05$], the TAAR1 KO group exhibited a trend toward greater CPP compared to WT group (595 ± 48 versus 413 ± 57 sec). Figure 3B illustrates that neither WT nor TAAR1 KO mice that were conditioned with 1.0 mg/kg methamphetamine showed a preconditioning bias to either compartment. Two-way ANOVA of time showed no main effects of compartment [$F(1,20)=3.6$; $p > 0.05$], genotype [$F(1,20)=0.5$; $p > 0.05$] or compartment $\times$ genotype interaction [$F(1,20)=0.01$; $p > 0.05$].

To further investigate the role of TAAR1 in conditioned reward, separate groups of mice were used to evaluate CPP induced by 1.0 mg/kg methamphetamine under sub-optimal conditions. Post-conditioning test 1 following only 2 methamphetamine conditioning sessions revealed that CPP was induced in the TAAR1 KO, but not in the WT mice (Figure 4A). Although two-way ANOVA revealed a significant effect of methamphetamine dose [$F(1,42)=18$; $p < 0.001$] but no main effect of genotype [$F(1,42)=1.4$; $p > 0.05$] and no methamphetamine dose $\times$ genotype interaction [$F(1,42)=1.7$; $p > 0.05$], further analysis by Bonferroni t-tests revealed a significant difference in the magnitude of CPP between WT and TAAR1 KO mice (*: $p < 0.05$) and between saline- and methamphetamine-conditioned mice (#: $p < 0.05$). Following 4 methamphetamine conditioning sessions, CPP during post-conditioning test 2 revealed that both the WT and TAAR1 KO mice acquired place preference (Figure 4B). Two-way ANOVA revealed a significant effect of methamphetamine dose [$F(1,42)=14$; $p < 0.001$], but no main effect of genotype [$F(1,42)=0.02$; $p > 0.05$] and no methamphetamine dose $\times$ genotype interaction [$F(1,42)=0.01$; $p > 0.05$]. Bonferroni t-tests revealed a significant difference in the magnitude of CPP between saline- and methamphetamine-conditioned mice (#: $p < 0.05$) for both genotypes.

Exposure to conditioning compartments in the absence of drug for 8 sessions resulted in a gradual decline in the difference in time spent between the drug- and saline-paired compartments for both genotypes (Figure 4C). Compared to the WT mice, CPP in the TAAR1 KO mice shifted the extinction curve upward. Two-way RM ANOVA of CPP revealed a significant effect of genotype [$F(1,90)=4.1$; $p < 0.05$] and a significant effect of time [$F(3,90)=2.8$; $p < 0.05$]. Reinstatement by a challenge injection of methamphetamine (0.56 mg/kg) following extinction increased the magnitude of CPP in both WT and TAAR1 KO mice, similar to place preference during the 1st and 3rd extinction sessions. Methamphetamine-induced reinstatement was not significantly different between genotypes (Student's t-test; $p > 0.05$).

3.3.2 Morphine-induced CPP—Figure 5 illustrates the magnitude of CPP produced by morphine (5 and 15 mg/kg) in separate groups of naïve mice. As the conditioning dose of morphine increased from 0 to 15 mg/kg, the difference in time spent between the drug- and saline-paired compartments increased. Unlike methamphetamine, morphine did not induce place preference in the TAAR1 KO mice in post-conditioning test 1. In post-conditioning test 2, the higher dose of morphine (15 mg/kg) induced CPP in both WT and TAAR1 KO mice. Two-way ANOVA revealed a significant effect of morphine dose [$F(2,34)=4$; $p < 0.05$], but no effect of genotype [$F(1,34)=0.01$; $p > 0.05$]. Bonferroni t-tests revealed a significant difference in CPP between saline- and morphine- (15 mg/kg) conditioned mice for both genotypes (#: $p < 0.05$). There was no significant difference in CPP between WT and TAAR1 KO mice following extinction or reinstatement by 3 mg/kg morphine (data not shown).

4. Discussion

It has been previously observed that exposure to one amphetamine injection can produce heightened locomotor activity responses in mice lacking the TAAR1 gene compared to their wild-type litter mates (Wolinsky et al. 2007; Lindemann et al. 2008). In this study, repeated amphetamine exposure produced sensitized behavioral responses that were more robust in TAAR1 KO mice compared to behavior in WT mice. This outcome was replicated following repeated methamphetamine exposure in the locomotor activity assay. Further, repeated methamphetamine exposure during conditioning in the place preference paradigm induced CPP in TAAR1 KO mice at an earlier time-point compared to WT mice. These findings provide in vivo evidence of a role for TAAR1 in mechanisms that regulate

behavioral sensitization and reward. In morphine CPP however, there appeared to be no difference between genotypes, suggesting that TAAR1 may be involved in dopamine signaling that is specifically mediated by psychostimulants rather than overall changes in the dopamine system that are not induced by psychostimulants, such as morphine.

Results of the locomotor activity experiments indicate that acute administration of lower test doses (1 and 3 mg/kg) of *d*-amphetamine produced greater psychomotor stimulating effects in TAAR1 KO mice compared to WT mice, and this finding generalized to methamphetamine in independent groups of mice. Further, chronic administration of escalating doses of either *d*-amphetamine or methamphetamine also produced greater effects in separate groups of TAAR1 KO mice compared to WT mice. These data are in agreement with previous findings by Wolinsky et al. (2007) in which TAAR1 KO mice were reported to display an enhanced sensitivity to the psychomotor-stimulating effect of 1 mg/kg *d*-amphetamine; by Lindemann et al. (2008) in which a different TAAR1 KO mouse line exhibited increased locomotor responses to a single injection of either 1 mg/kg or 2.5 mg/kg *d*-amphetamine compared to WT mice; and by Revel et al. (2011) in which selective activation of TAAR1 by the novel agonist, RO5166017, blocked cocaine-induced hyperlocomotion.

In CPP experiments, the magnitude of CPP achieved was similar between the two genotypes following 4 drug conditioning sessions. However, the finding that TAAR1 KO mice exhibited CPP under sub-optimal conditions, i.e. after 2 methamphetamine conditioning sessions, whereas the WT mice did not suggests that TAAR1 might play a role in modulating the rewarding effects of methamphetamine. In addition, the delay to extinguish CPP in the TAAR1 KO mice, compared to the WT mice suggests that the conditioned rewarding effect of methamphetamine is greater in TAAR1 KO mice. This augmented CPP in TAAR1 KO mice could be attributed to differences in learning and memory processes. CPP is a task that involves classical conditioning, and is dependent on both the salience of the unconditioned stimulus (methamphetamine), as well as associative learning processes (see Bardo and Bevins, 2000 for review) in which neural substrates involved in memory including the hippocampus and basolateral amygdala nuclei are recruited (Rademacher et al. 2010; Shen et al. 2006). Therefore, the finding that TAAR1 KO mice acquired CPP earlier than WT mice and retained CPP longer than WT mice could be interpreted also to suggest that TAAR1 might be involved in regulating learning and memory processes. Along these lines, Borowsky et al. (2001) have reported the occurrence of *TAAR1* mRNA in mouse hippocampus and amygdala and we have observed in our laboratory widespread expression of TAAR1-positive neurons in rhesus monkey hippocampus (unpublished findings) although Lindemann et al. (2008) did not report TAAR1 expression in mouse hippocampus.

The findings that methamphetamine generated augmented psychomotor stimulating and conditioned rewarding responses in TAAR1 KO mice compared to WT mice could be explained by previous observations by Wolinsky et al. (2007) in which TAAR1 KO mice exhibited significantly greater levels of extracellular dopamine (~2-fold) in the dorsal striatum at 30 to 60 min following *d*-amphetamine administration, when compared to WT mice. Furthermore, TAAR1 activation by *p*-tyramine in WT mice attenuates dopamine neuron firing rates in the ventral tegmental area (Bradaia et al. 2009; Lindemann et al. 2008). This suggests that in WT mice TAAR1 activation by *d*-amphetamine and methamphetamine could result in similar attenuation of dopamine neuron firing rates whereas TAAR1 KO mice however could maintain higher dopamine neuron firing rates, thereby generating relatively higher locomotor responses upon exposure to *d*-amphetamine and methamphetamine. In light of this, it is likely that the earlier onset and persistence of place preference in TAAR1 KO mice could be due to increased levels of dopamine release in TAAR1 KO mice upon methamphetamine exposure during conditioning sessions. Along

these lines, it has been demonstrated that the magnitude of drug-induced place preference is related to the degree of dopamine receptor activation when exposed to the drug-paired environment, as determined by PET imaging (Schiffer et al. 2009). In this way enhanced dopamine receptor activation, in TAAR1 KO mice compared to WT mice, upon exposure to the methamphetamine-paired compartment (cue-induced dopamine release) during testing could result in an earlier onset and more persistent CPP.

In contrast to methamphetamine, no differences in morphine-induced CPP between WT and TAAR1 KO mice were observed, suggesting that the function of TAAR1 might be related to changes in dopaminergic activation induced specifically by methamphetamine and not to overall changes in the dopamine system like that induced by morphine. Several studies have reported putative dopamine-independent mechanisms that may be involved in morphine-induced behaviors. For example, mice lacking the ability to synthesize dopamine are able to display robust CPP induced by morphine (Hnasko et al. 2005) and pretreatment with dopamine receptor antagonists, including haloperidol, have been shown to inhibit amphetamine-, but not morphine-induced CPP (Mackey and van der Kooy, 1985). Further, morphine-induced increases in locomotor activity are also thought to occur via mechanisms that do not appear to require dopamine (Kalivas et al. 1983). Given the role of TAAR1 in regulating dopamine neuron firing activity and subsequent dopamine release, these studies complement our finding that morphine-induced CPP was normal in TAAR1 KO mice compared to WT mice and suggests that TAAR1 might have a less critical role in mediating the conditioned rewarding effects of morphine.

Aside from this pharmacologically-induced phenotype and the observation that TAAR1 KO mice display a deficit in pre-pulse inhibition (Wolinsky et al. 2007), no other overt phenotype has yet been reported in mice lacking TAAR1, suggesting that TAAR1 might be nonessential to normal behaviors and physiological responsiveness. The recent observation that dogs and other carnivoran carnivores (but not other mammals) lack a functional *TAAR1* gene shows that the loss of TAAR1 is well-tolerated despite being under considerable evolutionary pressure to be conserved (Vallender et al. 2010). Nevertheless, our *in vivo* findings that the psychostimulant and rewarding effects of methamphetamine are augmented in mice lacking the TAAR1 gene indicate some regulatory role of TAAR1 in dopamine release by amphetamine-based stimulants. These findings further complement previous work showing that methamphetamine triggers changes in dopamine transporter kinetic function and internalization (Xie and Miller, 2009b) as well as studies describing how TAAR1 might exert an inhibitory tone on dopamine neurons to inhibit firing rates (Lindemann et al. 2008; Bradaia et al. 2009; Revel et al. 2011). It may also be the case that TAAR1 either directly interacts with dopamine receptors through heterodimerization, or indirectly via cooperative or competitive intracellular signaling cascades to modulate the dopamine system. Indeed, there is direct evidence for receptor-receptor physical interactions between TAAR1 and the dopamine D2 receptor (Espinoza et al. 2011), and it has also been shown that TAAR1 signaling is attenuated by D2 dopamine receptor activation (Xie et al. 2008). Accordingly, this study provides further support for the hypothesis that TAAR1 is a modulator of the dopamine system and the biochemical cascades triggered by amphetamine-like psychostimulants and points to a putative pharmacological target for the development of compounds that can diminish the rewarding effects of methamphetamine.

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Highlights

- TAAR1 KO mice exhibit greater amphetamine- and methamphetamine-induced locomotor activity compared to WT mice.
- TAAR1 KO mice achieve methamphetamine-induced CPP earlier than WT mice.
- There was no significant difference between KO and WT mice in morphine-induced CPP.
- TAAR1 may have a selective role in the reward-related behaviors of psychostimulants.
- Novel receptor family with putative role in rewarding effects of drugs of abuse.

Figure 1. Total distance travelled during a 60-min period immediately following d-amphetamine or methamphetamine (1, 3 or 5 mg/kg; n=15 per dose, per genotype) or saline (n=20 per genotype) injections; ** $p < 0.01$, * $p < 0.05$ comparing WT vs. TAAR1 KO mice.

Figure 2.

Total distance travelled during a 60-min period following administration of escalating doses of d-amphetamine or methamphetamine (0.5 to 6 mg/kg; n=4-5 per dose, per genotype). *** $p < 0.001$, * $p < 0.05$ comparing WT vs. TAAR1 KO mice.

Figure 3.

a) Methamphetamine dose-response curve showing the magnitude of CPP following place conditioning with methamphetamine (0.3 – 3.0 mg/kg; $n = 9-12$ per dose, per genotype). CPP score is the difference in time spent between the drug- and saline-paired compartments. Points above “Veh” represent data from control subjects that were administered saline during each conditioning session. b) Time spent in compartments during the preconditioning test for the group of mice that received the intermediate methamphetamine dose (1.0 mg/kg). # $p < 0.05$: Saline vs. methamphetamine conditioning.

Figure 4.

CPP tests following conditioning with methamphetamine (1.0 mg/kg; n=15-16 per genotype) or saline (n=8 per genotype) a) After only 2 methamphetamine conditioning sessions: Post-conditioning test 1 and b) After 4 methamphetamine conditioning sessions: Postconditioning test 2; c) Extinction of methamphetamine-induced CPP achieved by exposure to compartments in the absence of methamphetamine or saline injections and reinstatement to place preference in response to a priming injection of methamphetamine (0.56 mg/kg; i.m.). * $p < 0.05$; WT vs. TAAR1 KO; # $p < 0.05$: Saline vs. methamphetamine conditioning.

Figure 5. CPP induced by morphine 5 and 15 mg/kg (n=9 per dose, per genotype) after A) 2 saline and 2 morphine conditioning sessions and B) 4 saline and 4 morphine conditioning sessions. # $p < 0.05$: Saline vs. morphine conditioning.